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INHIBITION OF SWEET POTATO β-AMYLASE BY A POLYCATION

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ABSTRACT

A novel polycation was synthesized. Inhibition of partially purified sweet potato β-amylase by the polycation was studied. Fifty per cent inhibition was obtained at 6.5 mM. Kinetic studies indicate that inhibition was noncompetitive and reversible, and K_i was 7.3 mM. The Hill coefficient was 1, suggesting that the polycation interacts with the enzyme non-cooperatively. Thermodynamic studies on enzyme–polycation complex indicate that the reaction is exothermic. The negative value of ΔS indicates that the enzyme molecule undergoes conformational change during interaction with the polycation. The polycation also inhibits β-amylase derived from maize, wheat, barley and pea.

INTRODUCTION

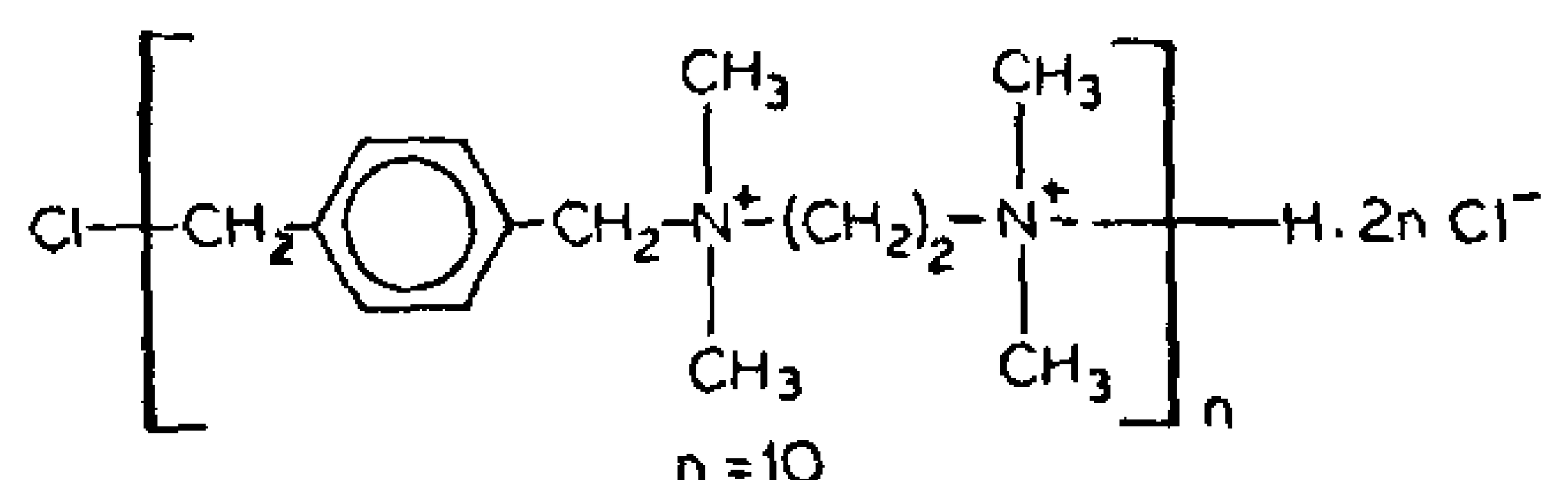
β-AMYLASE (α, 1–4 glucan maltohydrolase EC 3.2.1.2) catalyses the liberation of maltose from nonreducing ends of α, 1–4 glucan. The interaction of enzyme with substrates, products and inhibitors has been investigated by kinetic and spectroscopic methods[1]. Inhibition of soybean β-amylase by glucose[2], maltose[3] and cyclohexaamylase[4] have been demonstrated by kinetic studies.

Biologically important polyamines such as putrescine, spermidine and spermine are cationic substances, and have been shown to interact with DNA and RNA and to affect the activities of several enzymes[5–8]. It was reported that synthetic polycations also strongly interact with nucleic acids[9]. Recent studies with a polycation showed that it was a potential inhibitor of mammalian amylases[10,11]. The present investigation was undertaken to study the inhibitory action of a synthetic polycation on partially purified sweet potato β-amylase.

MATERIALS AND METHODS

Sweet potato β-amylase was purified according to the procedure of Hegde *et al.*[12] The enzyme from wheat, maize, barley and peanut was extracted according to Sharma *et al.*[13]

α,α'-Dichloro-p-xylene (XDC) was purchased from AG Switzerland; *N,N,N',N'*-tetramethylethylenediamine (TEMED) from BDH, England; and soluble starch and dimethyl formamide (DMF) from BDH, Bombay. The polycation shown below was synthesized according to the procedure of Rembaum and coworkers.[14,15]



The polycation was homogeneous. All other chemicals used were of analytical grade.

Enzyme assay

Enzyme activity was measured according to the method of Bernfeld[16] using dialysed starch as substrate. The amylase activity is expressed in terms of micromoles of reducing sugar liberated by 1 ml of enzyme per minute at room temperature. Protein concentration was determined according to Lowry *et al.*[17] using bovine serum albumin as standard.

Effect of polycation on plant amylases

Table 1 shows that the polycation inhibits β-amylase from maize, wheat, barley and pea to different extents in a concentration-dependent manner.

Detailed inhibition studies were carried out with sweet potato β-amylase. Different concentrations of polycation with 0.5 ml of enzyme and 0.5 ml of 1%

Table 1 *Effect of polycation on activity of plant amylases*

Inhibitor conc. (mM)	Inhibition (%)			
	Maize	Wheat	Barley	Pea
2	16.22	31.26	35.24	41.03
4	48.76	51.57	55.61	48.72
6	70.28	70.32	70.12	54.52
8	70.28	76.57	88.24	—

starch in 0.01 M acetate buffer, pH 4.8, were incubated at room temperature for 3 min and the residual activity was measured as absorbance at 540 nm in a Hitachi spectrophotometer. From the inhibition data a linear Hill plot of log $(V_0-V_i)/V_i$ vs log polycation concentration was plotted according to Johnson *et al.*[18] The type of enzyme inhibition by polycation was determined according to the method of Lineweaver and Burk[19] and the apparent inhibitor constant (K_i) was determined from a Dixon plot[20]. The Lineweaver–Burk plot was analysed by least square analysis[21]. Reversibility of polycation was studied by incubating the different concentrations of enzyme with two fixed concentrations of polycation and plotting the data in an Ackermann and Potter plot[22]. Thermodynamic parameters of enzyme–polycation complex were studied at different temperatures (0–30°C) and enthalpy (ΔH), free energy change (ΔG) and entropy (ΔS) were calculated according to Taketa and Pogell[23]. The energy of activation was calculated from an Arrhenius plot[24].

RESULTS AND DISCUSSION

Fifty per cent inhibition of partially purified sweet potato β-amylase by polycation was obtained at 6.3 mM (figure 1A). This showed that the polycation is a potent inhibitor of sweet potato β-amylase. Maximum inhibition was obtained at 10 mM. The Hill plot (figure 1B) is a straight line with slope 1, indicating noncooperativity, which is associated with most systems involving allosteric interactions[25]. The Lineweaver–Burk plot (figure 2) shows that the inhibition by the polycation is noncompetitive. K_i was found to be 7.3 mM. The Ackermann and Potter plot (figure 3) indicates that the polycation binds reversibly. The effect of temperature on the formation of enzyme–polycation complex was analysed in terms of thermodynamic parameters by assuming that K_i values at different temperatures represent association constants for enzyme–polycation complex formation. This assumption allowed calculation of the enthalpy change ΔH involved in the formation of the complex from a plot of pK_i as a function of the reciprocal of absolute temperature (figure 4A) as

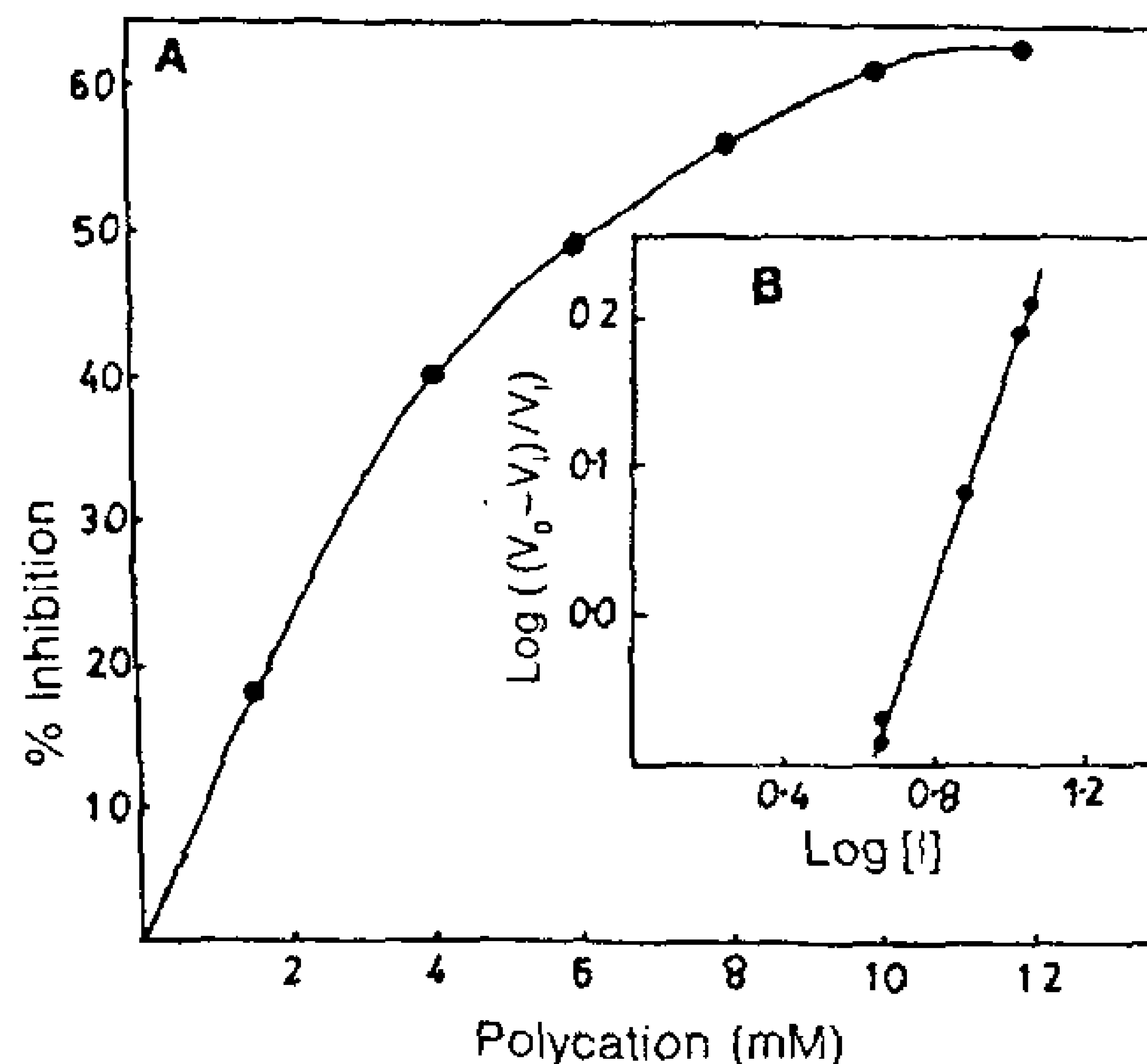


Figure 1. **A,** Inhibition of sweet potato β-amylase by polycation. **B,** Plot of $\log((V_0-V_i)/V_i)$ vs log polycation concentration. V_0 is enzyme activity without polycation and V_i is activity in presence of polycation.

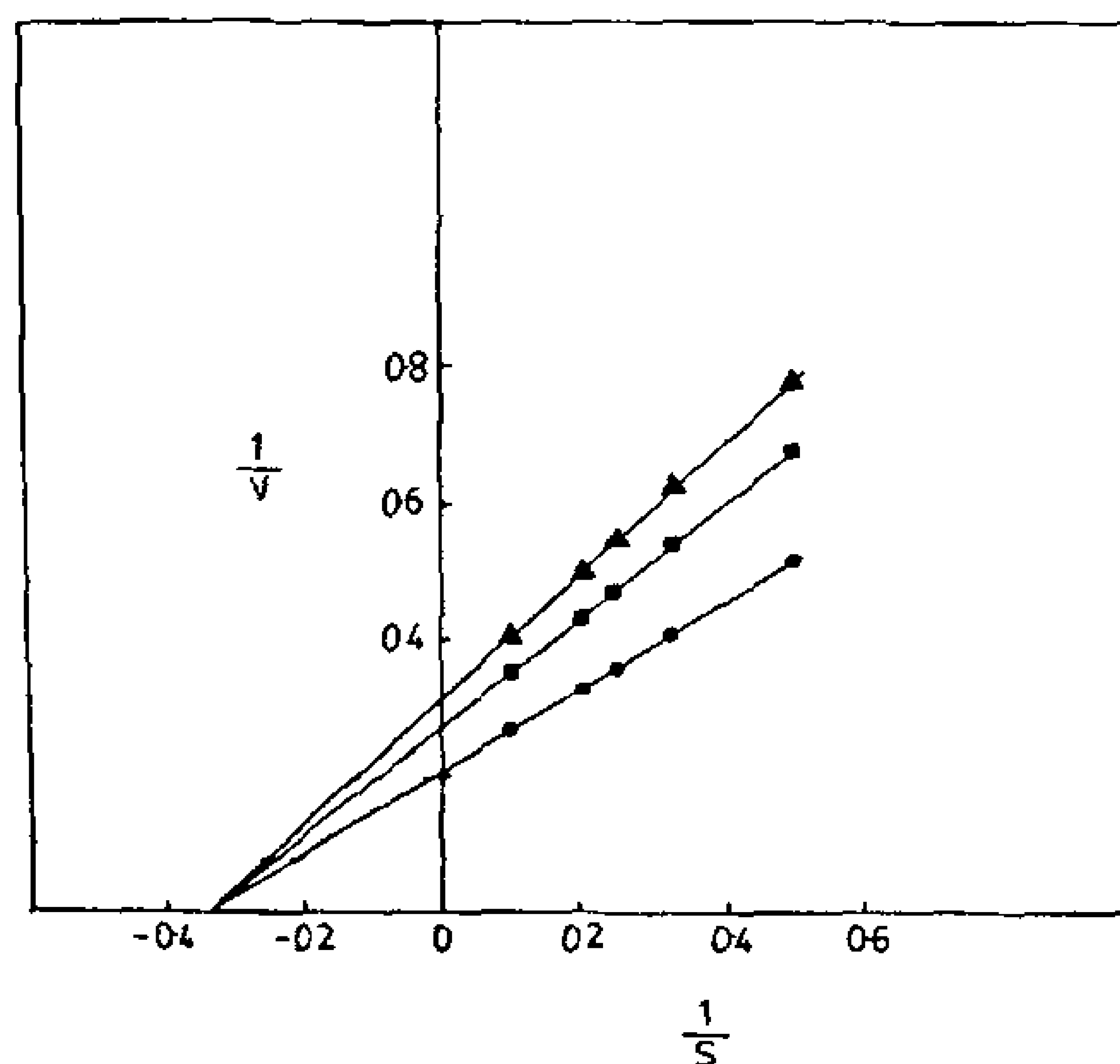


Figure 2. Lineweaver–Burk plot showing noncompetitive inhibition of sweet potato β-amylase by polycation. ●, No inhibitor; ■, 3 mM polycation; ▲, 5 mM polycation.

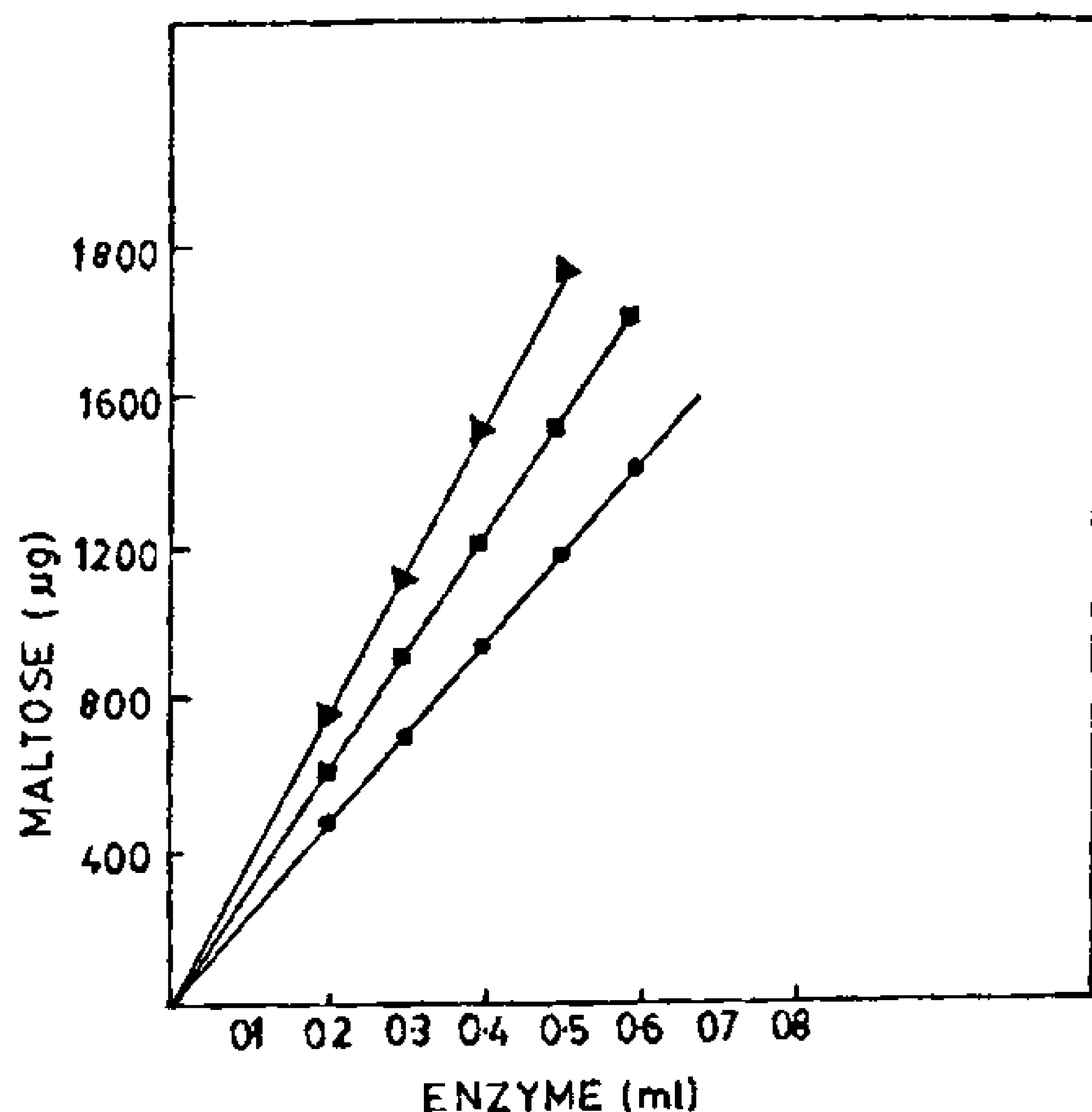


Figure 3. Ackermann and Potter plot. ▲, No inhibitor; ■, 2 mM polycation; ●, 5 mM polycation.

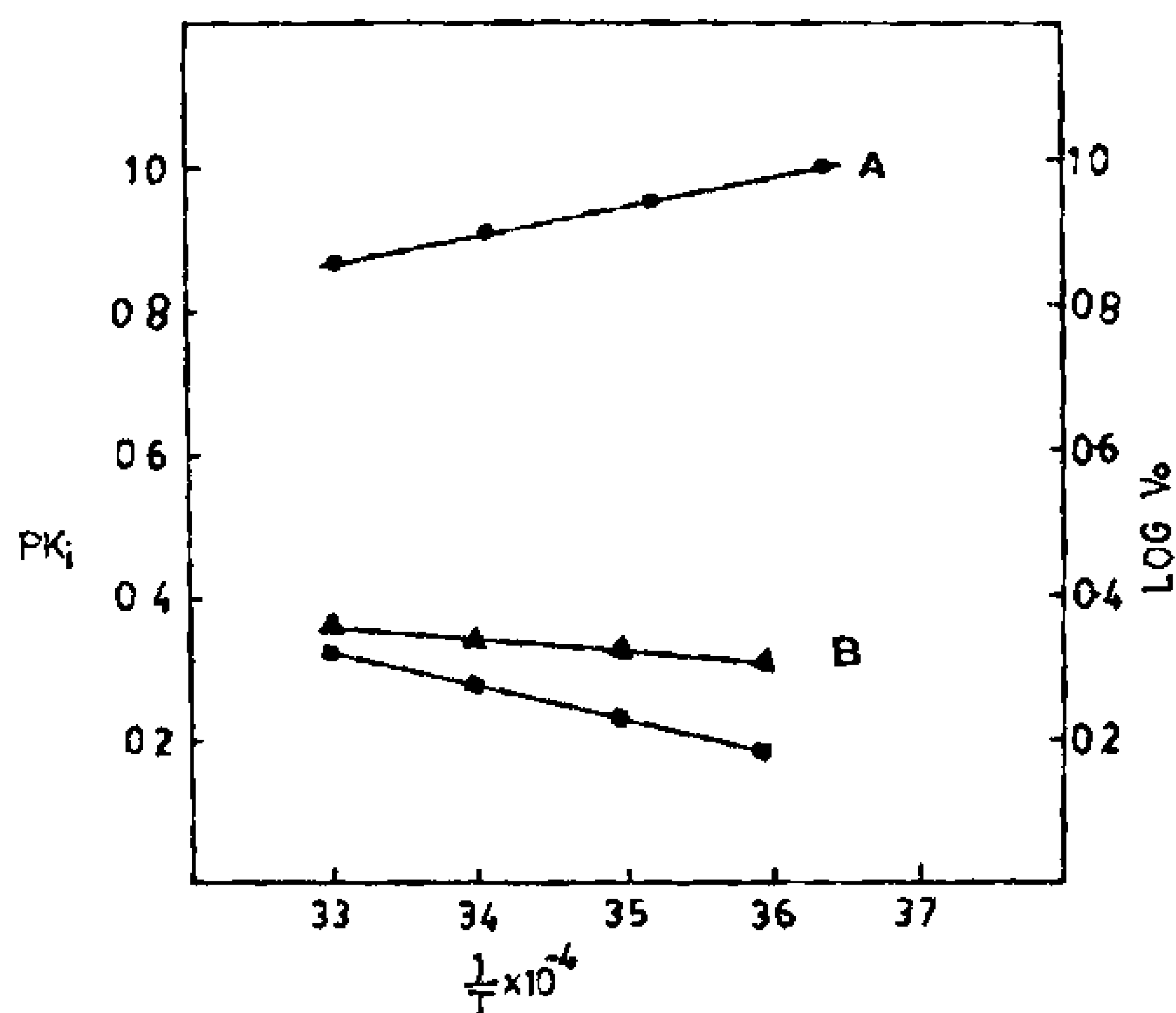


Figure 4. A, Van't Hoff plott. Values of K_i at different temperatures were obtained from Dixon plots. The molar enthalpy change ΔH (●) was calculated (plot of pK_i vs $\frac{1}{T} \times 10^{-4}$) from the slope according to the equation $\Delta H = -4.57 \times \text{slope}$. **B,** Arrhenius plot of log V_0 vs $\frac{1}{T} \times 10^{-4}$. Energy of activation E_a was calculated for enzyme (▲) and enzyme with polycation (■) from their respective slopes according to the equation $E_a = \text{slope} \times 2 \times 2.3$.

described by Taketa and Pogell[23]. The calculated ΔH value of -1091 cal/mol indicates the exothermic nature of the reaction. The free energy change (ΔG) and entropy change (ΔS) for enzyme–polycation complex formation were calculated using the following equations:

$$\Delta G = -RT \ln k_i \quad \text{and} \quad \Delta S = \frac{\Delta H - \Delta G}{T}.$$

Table 2 *Thermodynamic parameters for interaction between the polycation and sweet potato β-amylase*

Temp (°C)	ΔS (cal/deg/mol)	ΔG (cal/mol)
0	−2.180	1305.8
10	−2.401	1221.4
20	−2.569	1148.1
30	−2.660	1093.2

The results are summarized in table 2. The ΔS values were negative and remained constant throughout, indicating that the formation of inactive β-amylase–polycation complex involved marked conformational change in the enzyme. Energy of activation was calculated from figure 4B and is 506 cal/mol for enzyme alone and 920 cal/mol for enzyme plus polycation. The difference is required to break the noncovalent bonds of the enzyme–polycation complex.

Ikemiya[26,27] found that alkyldimethylbenzylammonium chloride was a potential inhibitor of β-amylase from malt, soybean and sweet potato. Pomeranz[28] reported that cetyl quaternary ammonium compounds were highly effective in inactivating amylases from pancreas, bacteria and fungi. Mulimani and Day[29] showed that the polycation inhibits *Bacillus subtilis* α-amylase; enzyme inhibition was achieved by electrostatic interaction between polycation and β-amylase. The quaternary nitrogen group of the polycation interacts electrostatically with essential anionic sites of the enzyme. The affinity of the polycation for the anionic sites of the enzyme has been shown to depend on the presence of the long-chain alkyl group.

Direct interaction between polyamines and protein kinases has been described, which might have led to conformational change in the binding site[30]. Inhibition of sweet potato β-amylase by polycation might also be due to a specific change induced by the binding of polycation. The positive quaternary nitrogen of polycation is specifically adapted to substrate binding region of β-amylase.

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1. Keiichi Nomura, Bunzo Mikami and Yuhei Morita, *J. Biochem.*, 1986, **110**, 1175.

2. Thoma, J. A., Spradlin, J. E. and Dygert, S., In: *The Enzyme*, 3rd edn, (ed.) P. D. Boyer, Academic Press, 1971, vol. 5, p. 115.
3. Misra, U. K. and French, D., *Biochem. J.*, 1960, **77**, 1.
4. Marshall, J. J., *Eur. J. Biochem.*, 1973, **33**, 494.
5. Tabor, C. W. and Tabor, H., *Annu. Rev. Biochem.*, 1976, **45**, 255.
6. Raina, A. and Janne, *J. Med. Biol.*, 1981, **59**, 269.
7. Karpetsky, T. P., Hieter, P. A., Frank, J. J. and Levy, C. C., *Mol. Cell. Biochem.*, 1977, **17**, 89.
8. Abraham, A. K. and Phil, A., *Trends Biochem. Sci.*, 1981, **6**, 106.
9. Mulimani, V. H., *Curr. Sci.*, 1985, **54**, 10.
10. Mulimani, V. H. and Gopal, M., *Indian J. Med. Res.*, 1988, (in press).
11. Mulimani, V. H. and Gopal, M., *Arogya—J. Health Sci.*, 1988, **14**, 88.
12. Hegde, M. V., Francis Roy and Joshi, J. N., *Prep. Biochem.*, 1979, **9**, 1, 71.
13. Sharma, C. B., Goel, H. and Irshad, M., *Phytochemistry*, 1978, **17**, 201.
14. Rembaum, A., Baumgartner, W. and Eisenberg, A. J., *Polym. Sci.*, 1968, **B6**, 159.
15. Noguchi, H. and Rembaum, A. J., *Polym. Sci.*, 1969, **7**, 383.
16. Bernfeld, P., *Methods Enzymol.*, 1955, **1**, 149.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
18. Johnson, F., Egring, H. and William, R. W., *J. Cell Comp. Physiol.*, 1942, **20**, 247.
19. Lineweaver, H. and Burk, D. J., *J. Am. Chem. Soc.*, 1934, **56**, 658.
20. Dixon, M., *Biochem. J.*, 1953, **55**, 170.
21. Schefler, W. C., *Statistics for the Biological Sciences*, Addison–Wesley, 1969.
22. Ackermann, W. W. and Potter, V. R., *Proc. Soc. Exp. Biol. Med.*, 1949, **72**, 1.
23. Taketa, K. and Pogell, B. H., *J. Biol. Chem.*, 1955, **240**, 651.
24. Whitaker, J. R., *Principles of Enzymology for Food Science*, Marcel Dekker, 1972, p. 332.
25. Atkinson, D. E., Halthway, J. A. and Smith, E. C., *J. Biol. Chem.*, 1965, **240**, 2682.
26. Ikemiya, M., *Bull. Inst. Chem. Res. Kyoto Univ.*, 1957, **35**, 81; *Chem. Abstr.*, 1958, **52**, 11998.
27. Ikemiya, M., *Bull. Inst. Chem. Res. Kyoto Univ.*, 1957, **35**, 89; *Chem. Abstr.*, 1958, **52**, 12946.
28. Pomeranz, Y., *Biochem. Biophys. Acta*, 1963, **73**, 105.
29. Mulimani, V. H. and Day, R. A., *Curr. Sci.*, 1981, **50**, 629.
30. Hathway, K., Hirota, T., Aquilera, G. and Catt, K., *J. Biochem.*, 1985, **260**, 32437.